# CLAIMS

I claim:

1. In combination, a shoe, elastic lace, means to form an endless loop in said lacing system, and means to tension said endless loop.

2. A method of lacing a shoe having an elastic tie, comprising the steps of:

passing said lace through eyelets to secure said shoe;

securing opposed ends of said lace to form said lace into an endless loop;

passing said secured opposed ends of said lace through tongue retainers; and

attaching said lace to an anchor secured to said shoe.

3. A shoe and method of lacing as shown, described and which may be imputed by those skilled in the art.

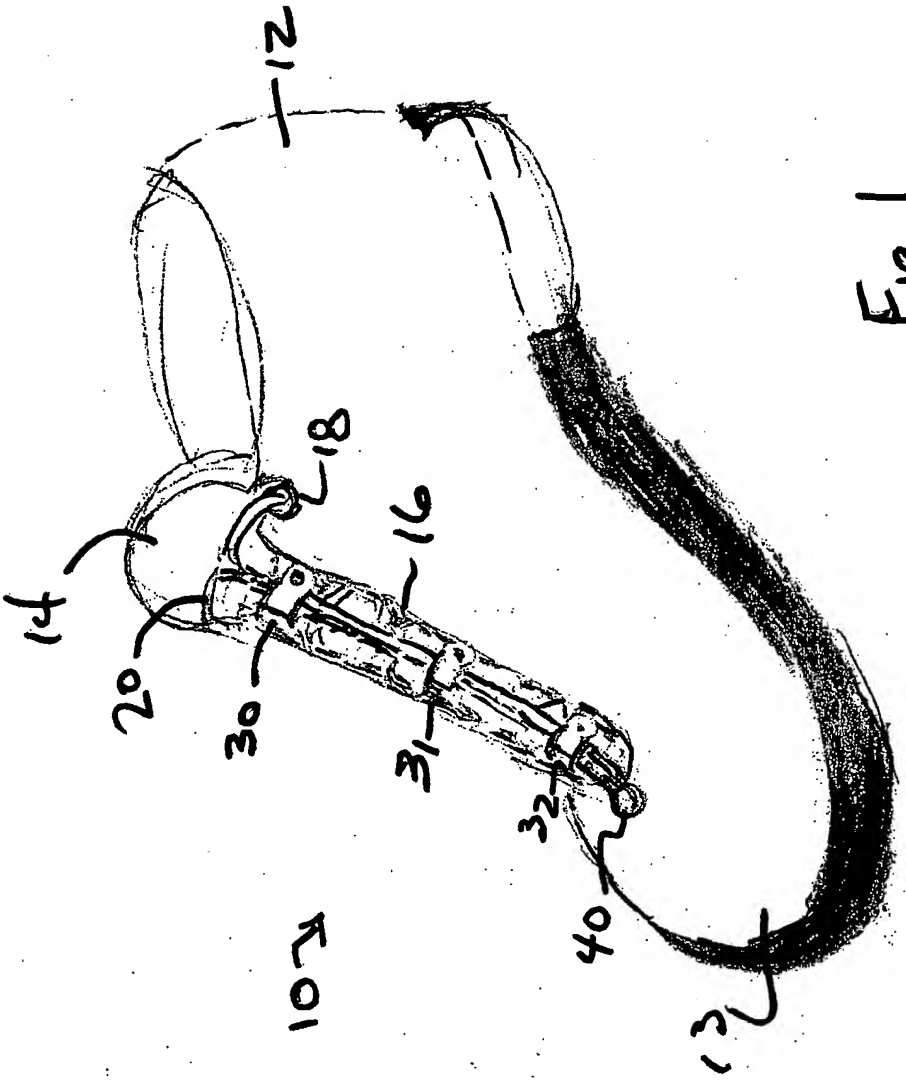



Fig 1

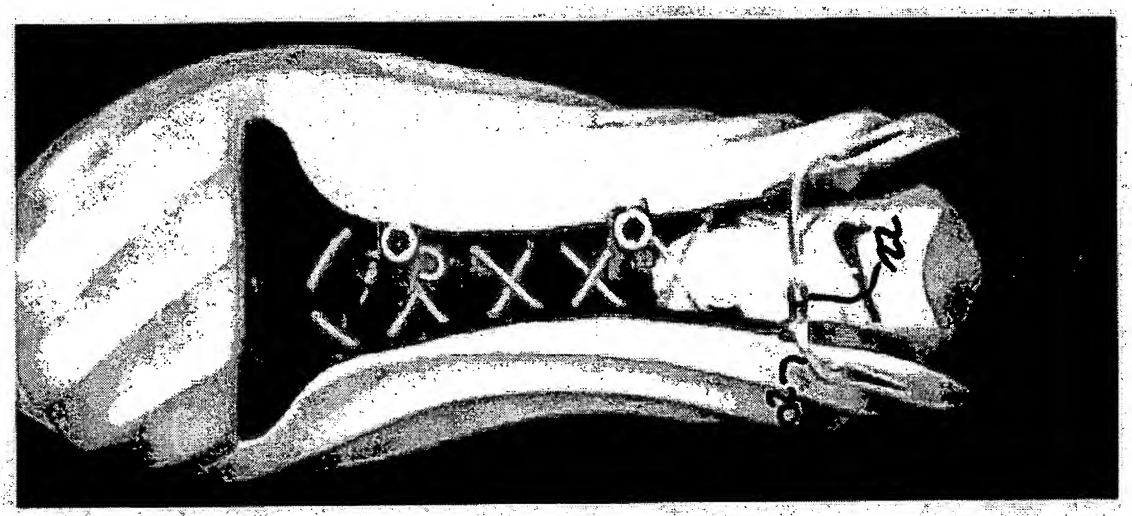


Fig 2

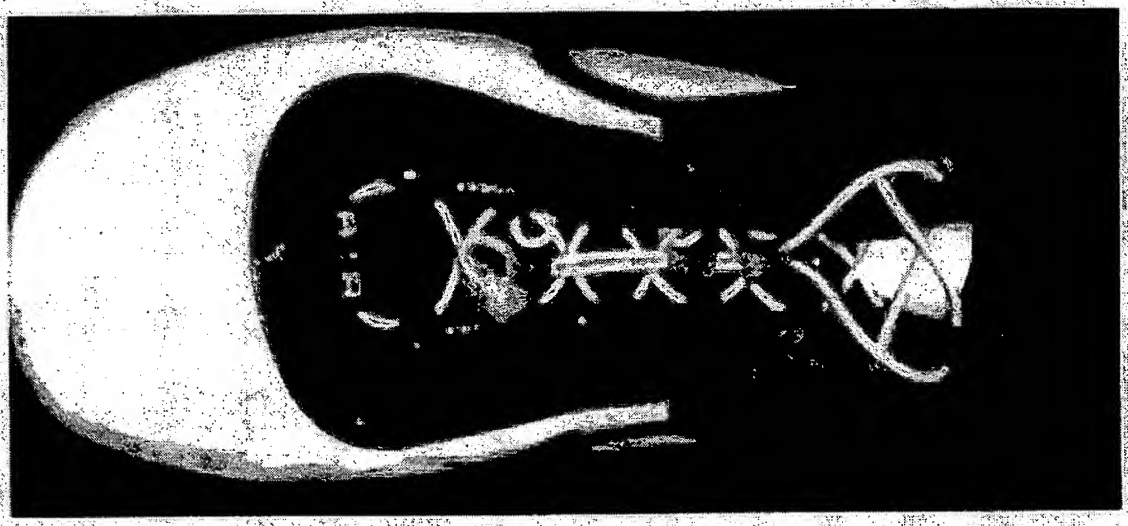


Fig 3

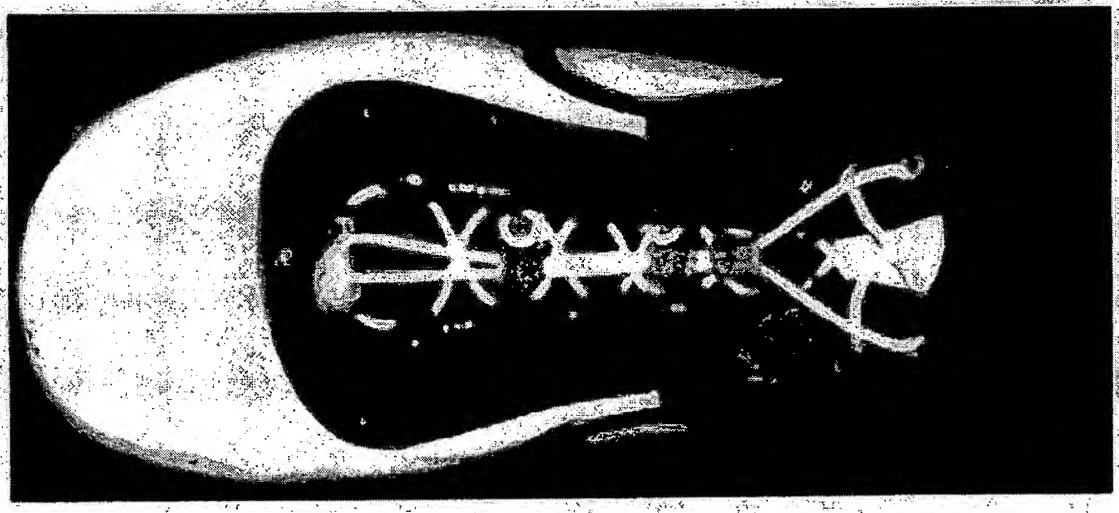


Fig 4

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) & 1.27(b))--INDEPENDENT INVENTOR

Docket Number **Miller-Laces**

Applicant or Patentee: Corey C. Miller

Serial or Patent No.: Herewith

Filed or Issued: Herewith

Title: Lacing System Using Elastic Tie

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in the application identified above.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign. grant, convey, or license any rights in the invention is listed below:

☒ No such person, concern, or organization exists.

☐ Each such person, concern or organization is listed below.

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

1 acknowledge thc duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF INVENTOR Corey C. Miller

Signature of inventor 

Date September 9, 2003

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

09/12/2003 SMINASS1 00000030 60501690

01 FC:2005 80.00 DA

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 489-267/69033

Aligned with exit opening 34 is a detector array 42 consisting of individual detectors 43. The detector array 42 may take many different forms, such as an array of photomultiplier tubes (PMTs), multiple window PMTs, position-dependant wire detectors, position/time-sensitive detectors, a photodiode array, an intensified photodiode array, charge-coupled devices (CCDs), intensified CCDs, an SIT or other video camera, or any other suitable optical-to-electrical transducer.

In any event, the detector array 42 is preferably a linear array, with the respective wavelength bands being incident upon a corresponding one of the detectors 43. Each detector 43 in the array receives the light incident upon it and generates a corresponding analog electrical signal. The electrical signals are then introduced to respective analog-to-digital converters (shown schematically at 44) which convert the incoming analog signals into corresponding digital signals. The digital signals are then delivered to a processor 46 that processes the digital signals to determine the constituents of the emissions spectra, as is described in more detail below.

Referring now to FIG. 3, there is shown a system 50 according to an alternative embodiment of the invention. In place of spectral analyzer 28, system 50 includes a tunable filter 52 that is interposed between lens 24 and a single detector 54, for example, a PMT. In one embodiment, tunable filter comprises a liquid crystal tunable filter (LCTF) that utilizes liquid crystals to continuously vary the retardance of individual filter stages, resulting in a narrow band filter that is electrically tunable over a wide spectral range. Alternatively, the tunable filter 52 may comprise an acousto-optical tunable filter. In any event, tunable filter is controlled by a suitable control unit 56 to vary the bandpass of the filter through the spectral range. At each bandpass, detector 54 receives fluorescence and generates a corresponding electrical output signal, which is converted to digital format by an analog-to-digital converter 58 and then introduced to processor 46 for processing.

Referring now to FIG. 4, there is shown a system 60 according to still another embodiment of the invention. System 60 includes a long pass dichroic mirror 62 placed in the light stream between the scanning optics 20 and the microscope lens 22. The dichroic mirror 62 is selected such that wavelengths corresponding to the fluorescence radiated by the specimen are reflected by the mirror 62, while the laser light from laser 14 passes through without being reflected. The reflected fluorescence is directed to a focusing lens 64, and then introduced to light guide 26 which delivers the light to spectral analyzer 28. As described above, spectral analyzer disperses the light and passes the light on to ADC 44, which converts the respective bands into digital signals and introduces the digital signals to processor 46.

System 60 is therefore suitable for use in connection with a non-descanned two-photon

microscope. By diverting the radiated fluorescence before it passes through the scanning optics 20, a signal of increased intensity is received by spectral analyzer 28, as compared with a signal that passes through scanning optics 20 and dichroic mirror 18 before being received by a spectral analyzer or detector. Thus, the dwell time at each pixel can be reduced as a result.

Referring now to FIG. 5, a system 70 is shown according to another embodiment of the invention. System 70 includes a light collector 72 that substantially surrounds specimen 12 and includes a reflective inner surface. Thus, light emitted by fluorescent dyes within specimen 12 are collected by the collector, regardless of the direction in which the light radiates. In one embodiment, collector 72 comprises an integrating sphere. Alternatively, collector 72 may be in the shape of an ellipsoid or other structure that substantially encompasses specimen 12 to collect a substantial amount of the light radiating from specimen 12, for example, an elliptical mirror. Collector 72 connects to light guide 26 to deliver the collected light to spectral analyzer 28.

Thus, in use of the various systems described above, laser light is directed by dichroic mirror 18, scanning optics 20, and focusing lens 22 to a region of specimen 12. The photons (either from a single-photon laser or from a multi-photon laser) excite the fluorescent dyes in the region, causing them to fluoresce. The entire emitted spectrum is received and processed simultaneously in certain of the illustrative embodiments to speed up the collection process. The spectral information is then processed to determine the amounts of each dye contributing to the emitted spectrum.

Referring now to FIG. 6, operation of processor 46 is described in greater detail. In one embodiment, processor 46 is programmed to execute a linear unmixing operation upon the incoming spectral data to approximate the quantities of each fluorescent dye that contributed to the emitted spectrum. As shown in FIG. 6, operation begins at step 100 by determining spectral characteristics for the respective individual fluorescent dyes. As is well known in the art, each of the fluorescent dyes emits a particular spectrum over a certain wavelength band and at certain varying intensities within that band (see FIGS. 7a-c which illustrate examples of emissions spectra for three different fluorescent dyes). At step 102, data relating to the probe spectra is recorded in processor memory for subsequent retrieval.

At step 104, processor 46 receives the measured imaging spectrum data from ADC 44 (see FIG. 7d), and retrieves the characteristic spectra for the various fluorescent dyes. At step 106, the measured spectrum is decomposed into the various component dye spectra. This can be accomplished in many different ways. In one embodiment, processor 46 generates a model spectrum from the individual templates, and compares the model spectrum with the actual recorded spectrum. The respective weights of each of the individual templates are then varied

to arrive at a close approximation of the actual spectrum. Then, at step 108, processor 46 determines the weights of each dye to quantitate the respective dye intensities.

In another embodiment, processor 46 is programmed to carry out principal component analysis (PCA) on the incoming data. As is well known in the art, PCA is a linear model which transforms the original variables of an emission spectrum into a set of linear combinations of the original variables called principal components, that account for the variance in the original data set. Suitable forms of PCA algorithms are disclosed in U.S. Patent Numbers 5,991,653 to Richards-Kortum et al., and 5,887,074 to Lai et al., the disclosures of which are hereby expressly incorporated by reference.

Preferably, system 10 uses a single excitation to excite the respective dyes (i.e., the dyes are pan-excited). In this manner, all of the dyes are excited at once by using a single wavelength. Thus, there is no need for using more than one wavelength, nor is there a need to take multiple images and overlay the respective pixel images to generate a complete image. Alternatively, system 10 may use multiple excitations, such as two excitations, to excite the dyes, especially in situations where it is desirable to cover a relatively broad span of dyes.

From the foregoing, it will be apparent to those skilled in the art that the present invention provides an efficient and reliable system for receiving and processing emissions spectra in connection with fluorescence microscopy. The system processes the emissions spectra to determine concentrations of plural fluorescent dyes in a particular spectrum, even where the wavelength bands of the dyes overlap.

While the above description contains many specific features of the invention, these should not be construed as limitations on the scope of the invention, but rather as one exemplary embodiment thereof. Many other variations are possible. Accordingly, the scope of the invention should be determined not by the embodiments illustrated, but by the appended claims and their legal equivalents.

What is claimed is:

1. A method of monitoring cellular activity in a cellular specimen, comprising:

applying a plurality of different excitable markers to the specimen;

applying light to the specimen from a multi-photon laser microscope to excite a region of the specimen and cause fluorescence to be radiated from the region by the markers in that region;

separating the fluorescence into wavelength bands using a spectral analyzer; and

detecting the fluorescence through an array of detectors, with each detector receiving one of the wavelength bands and generating a corresponding signal.

2. The method of claim 1, wherein separating the fluorescence includes using a grating.

3. The method of claim 1, wherein separating the fluorescence includes using a prism.

4. The method of claim 1, wherein separating the fluorescence includes using a liquid crystal tunable filter.

5. The method of claim 1, wherein separating the fluorescence includes using an acousto-optical tunable filter.

6. The method of claim 1, wherein applying a plurality of excitable markers includes applying a plurality of fluorescent probes to the specimen.

7. The method of claim 1, wherein detecting the fluorescence includes using a plurality of photomultiplier tubes.

8. The method of claim 1, wherein detecting the fluorescence includes using a plurality of high gain photomultiplier tubes.

9. The method of claim 1, wherein applying light to the specimen comprises applying light from a two-photon laser microscope.

10. A system for monitoring cellular activity in a cellular specimen that contains a plurality of excitable markers, the system comprising:

a laser microscope that is operative to excite the markers in a region of the specimen, wherein the markers in the region radiate fluorescence as a result;

a tunable filter that is operative to process the fluorescence and to pass a portion of the fluorescence, wherein the portion of the fluorescence is within a wavelength band that

depends on the setting of the filter; and

a detector that is operative to receive the processed fluorescence and to convert the fluorescence into a corresponding signal.

11. The system of claim 10, wherein the tunable filter comprises a liquid crystal tunable filter.

12. The system of claim 10, wherein the tunable filter comprises an acousto-optical tunable filter.

13. The system of claim 10, wherein the detector comprises a photomultiplier tube.

14. The system of claim 10, wherein the detector comprises a high-gain photomultiplier tube.

15. The system of claim 10, wherein the laser microscope comprises a multi-photon laser microscope.

16. A system for monitoring cellular activity in a cellular specimen that contains a plurality of excitable markers, the system comprising:

a multi-photon laser microscope that is operative to excite the markers in a region of the specimen, wherein the markers in the region radiate fluorescence as a result;

a deflector positioned relative to the specimen to deflect non-descanned fluorescence radiated by the markers;

a spectral analyzer operative to receive the deflected, non-descanned fluorescence from the deflector and to disperse the fluorescence; and

a detector array that is operative to receive the dispersed fluorescence from the spectral analyzer and to generate corresponding signals.

17. The system of claim 16, wherein the detector array comprises a plurality of photomultiplier tubes.

18. The system of claim 16, wherein the detector array comprises a plurality of high-gain photomultiplier tubes.

19. A method of monitoring cellular activity in a cellular specimen, comprising:

applying a plurality of different excitable markers to the specimen;

focusing light upon a region of the specimen from a laser microscope to excite the markers in the region and cause fluorescence to be radiated by the markers in the region;

separating the fluorescence into wavelength bands;

detecting the fluorescence through an array of detectors, with each detector receiving one of the wavelength bands and generating a corresponding signal; and

processing the signals from the detectors to calculate the quantities of each marker in the region.

20. The method of claim 19, wherein separating the fluorescence includes using a grating.

21. The method of claim 19, wherein separating the fluorescence includes using a prism.

22. The method of claim 19, wherein separating the fluorescence includes using a liquid crystal tunable filter.

23. The method of claim 19, wherein separating the fluorescence includes using an acousto-optical tunable filter.

24. The method of claim 19, wherein applying a plurality of excitable markers includes applying a plurality of fluorescent probes to the specimen.

25. The method of claim 19, wherein collecting the fluorescence includes using a plurality of photomultiplier tubes.

26. The method of claim 19, wherein collecting the fluorescence includes using a plurality of high gain photomultiplier tubes.

27. The method of claim 19, wherein applying light to the specimen comprises applying light from a two-photon laser microscope.

28. The method of claim 19, wherein processing the signals comprises performing linear unmixing on the signals.

29. A system for monitoring cellular activity in a cellular specimen that contains a plurality of excitable markers, the system comprising:

a laser microscope that is operative to excite the markers in a region of the specimen, wherein the markers in the region radiate fluorescence as a result;

a collector that at least substantially envelops the specimen to receive fluorescence from the markers;

a spectral analyzer connected to the collector to receive the fluorescence and process same to disperse the fluorescence; and

a detector that is operative to receive the dispersed fluorescence and to convert the fluorescence into corresponding signals.

30. The system of claim 29, wherein the collector comprises an integrating sphere.

31. The system of claim 29, wherein the detector comprises an array of photomultiplier tubes.

32. The system of claim 29, wherein the detector comprises an array of high-gain photomultiplier tubes.

33. The system of claim 29, wherein the laser microscope comprises a multi-photon laser microscope.

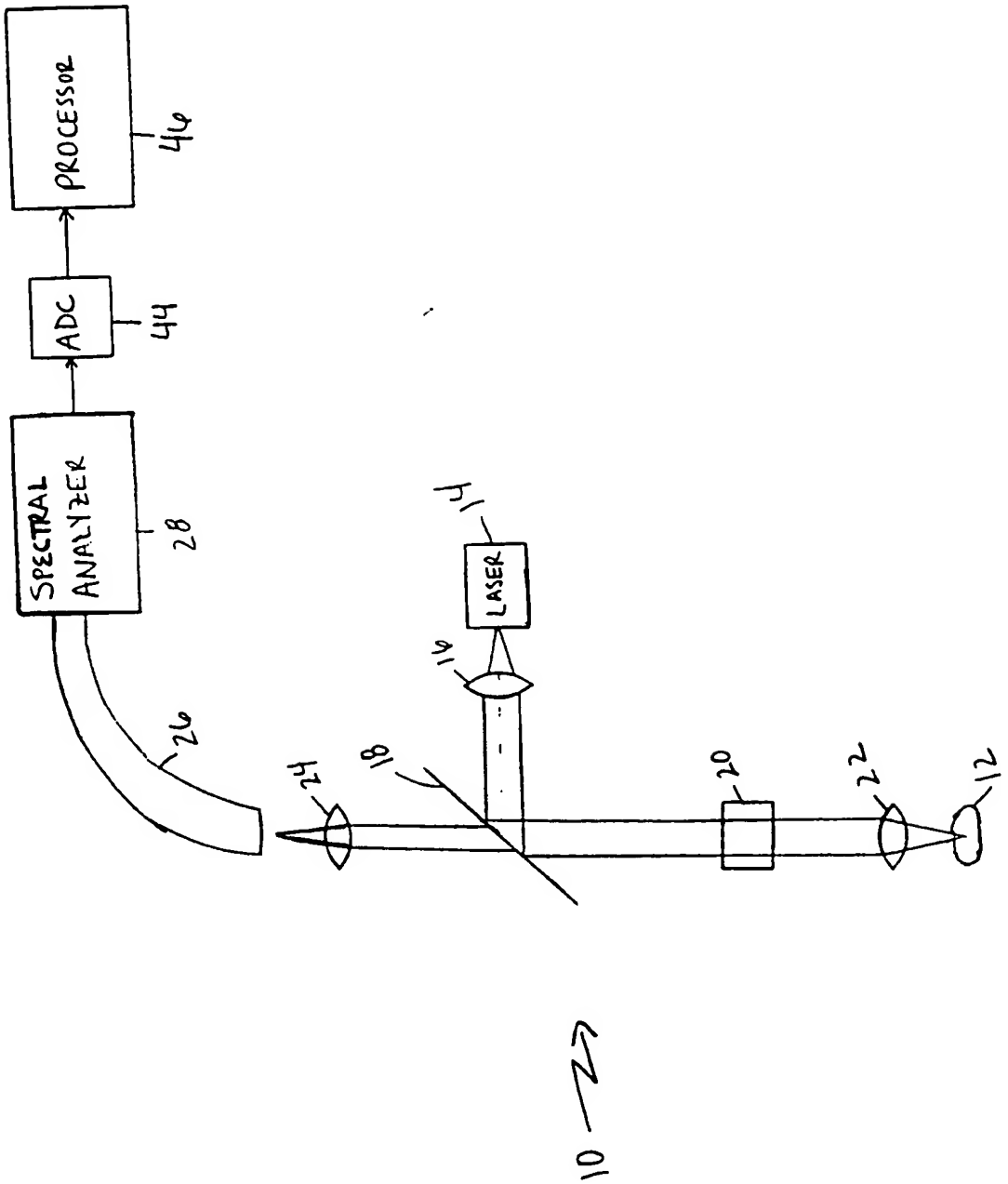

PROCESSOR
46
ADC
44
SPECTRAL ANALYZER
28
26
24
18
16
LASER
14
20
22
12
10

FIG. 1

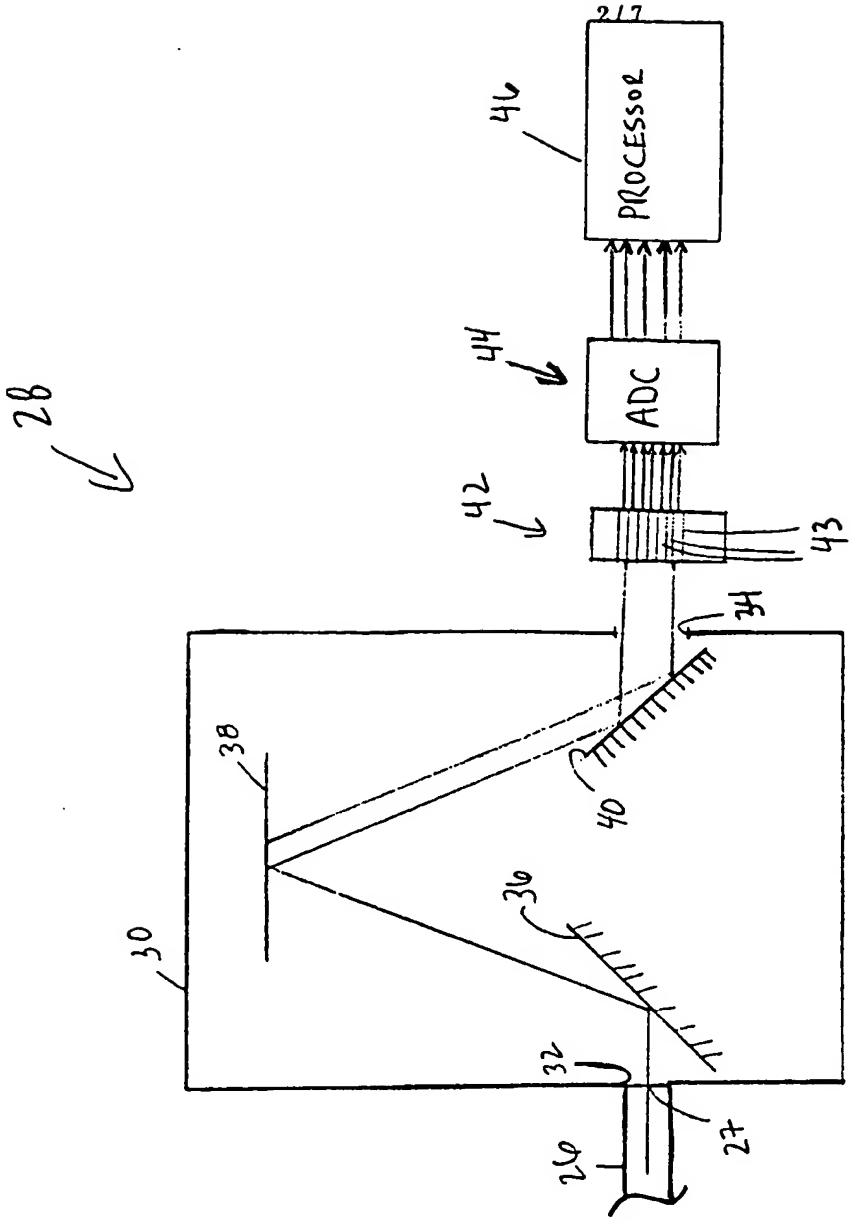



FIG. 2

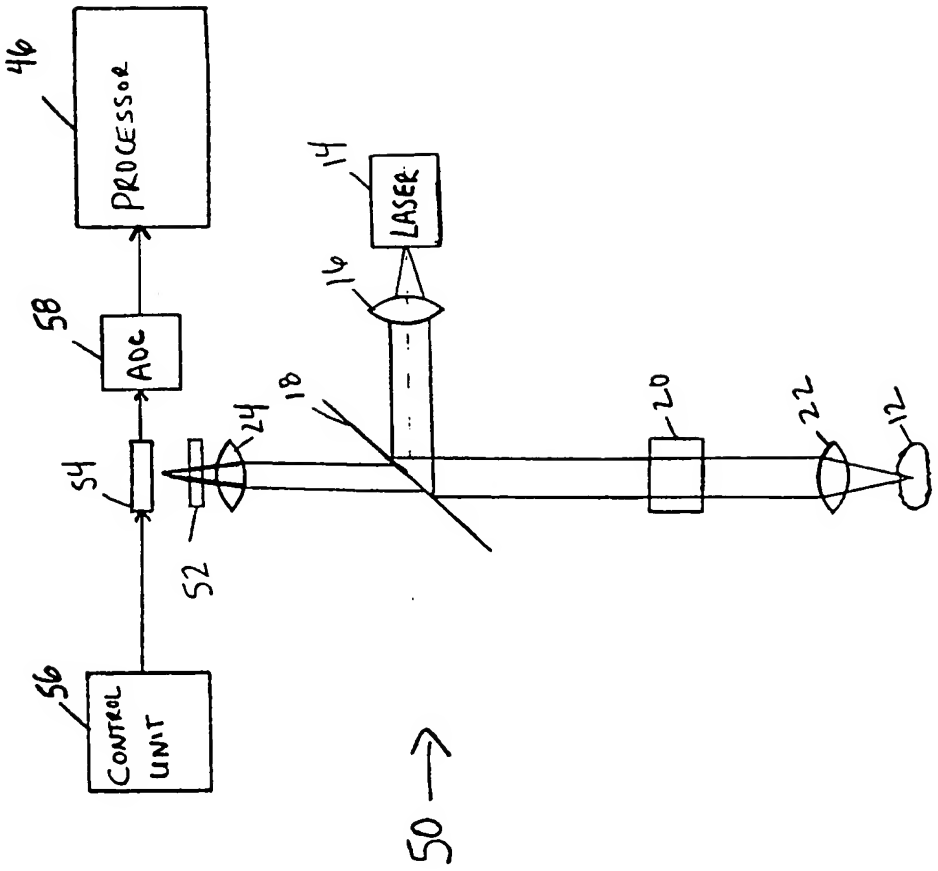

46
PROCESSOR
58
ADC
54
56
CONTROL UNIT
52
24
18
14
LASER
16
20
22
12
50

FIG. 3

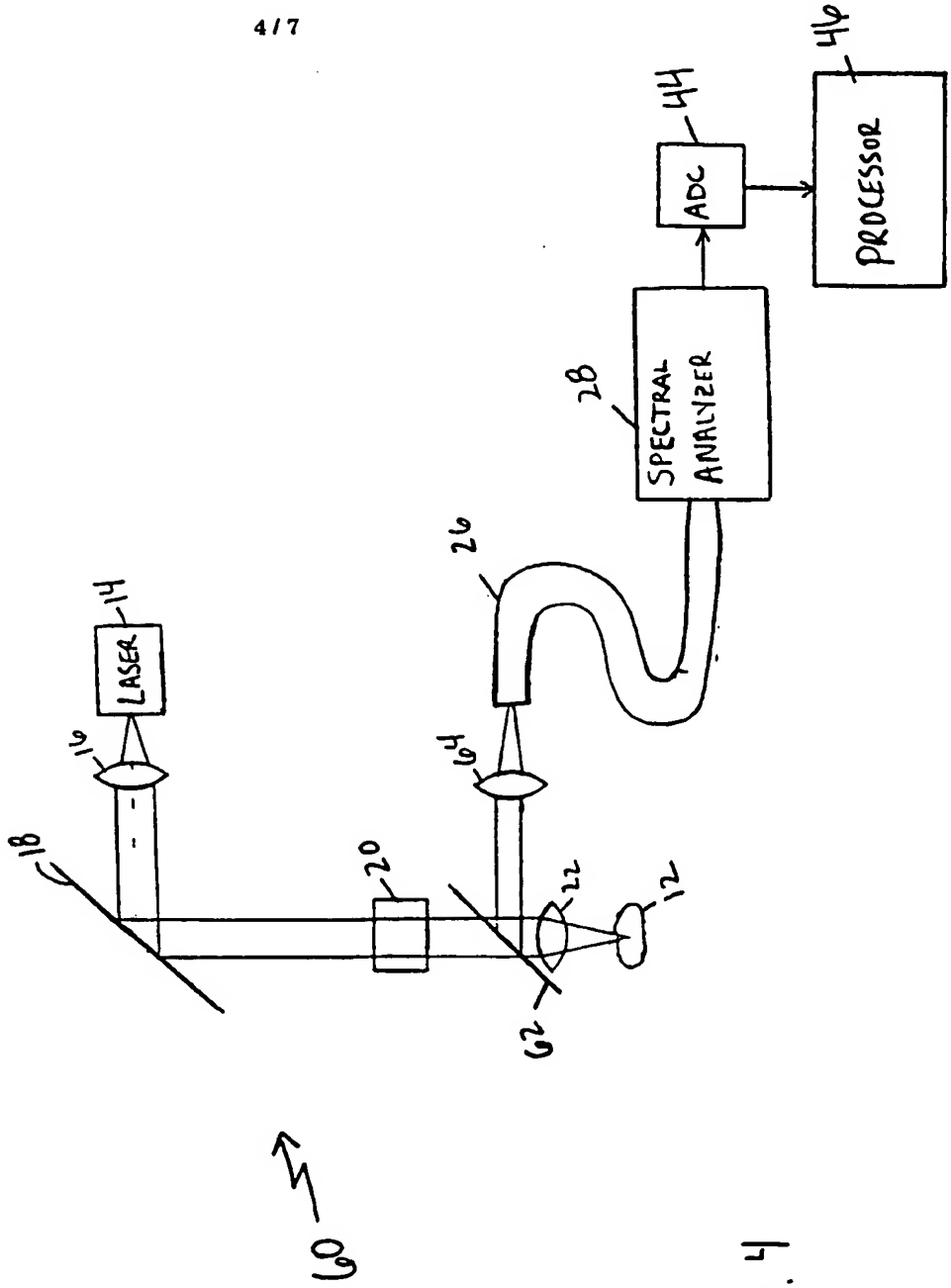

46
PROCESSOR
44
ADC
28
SPECTRAL ANALYZER
26
14
LASER
16
16'
18
20
22
12
62
60

FIG. 4

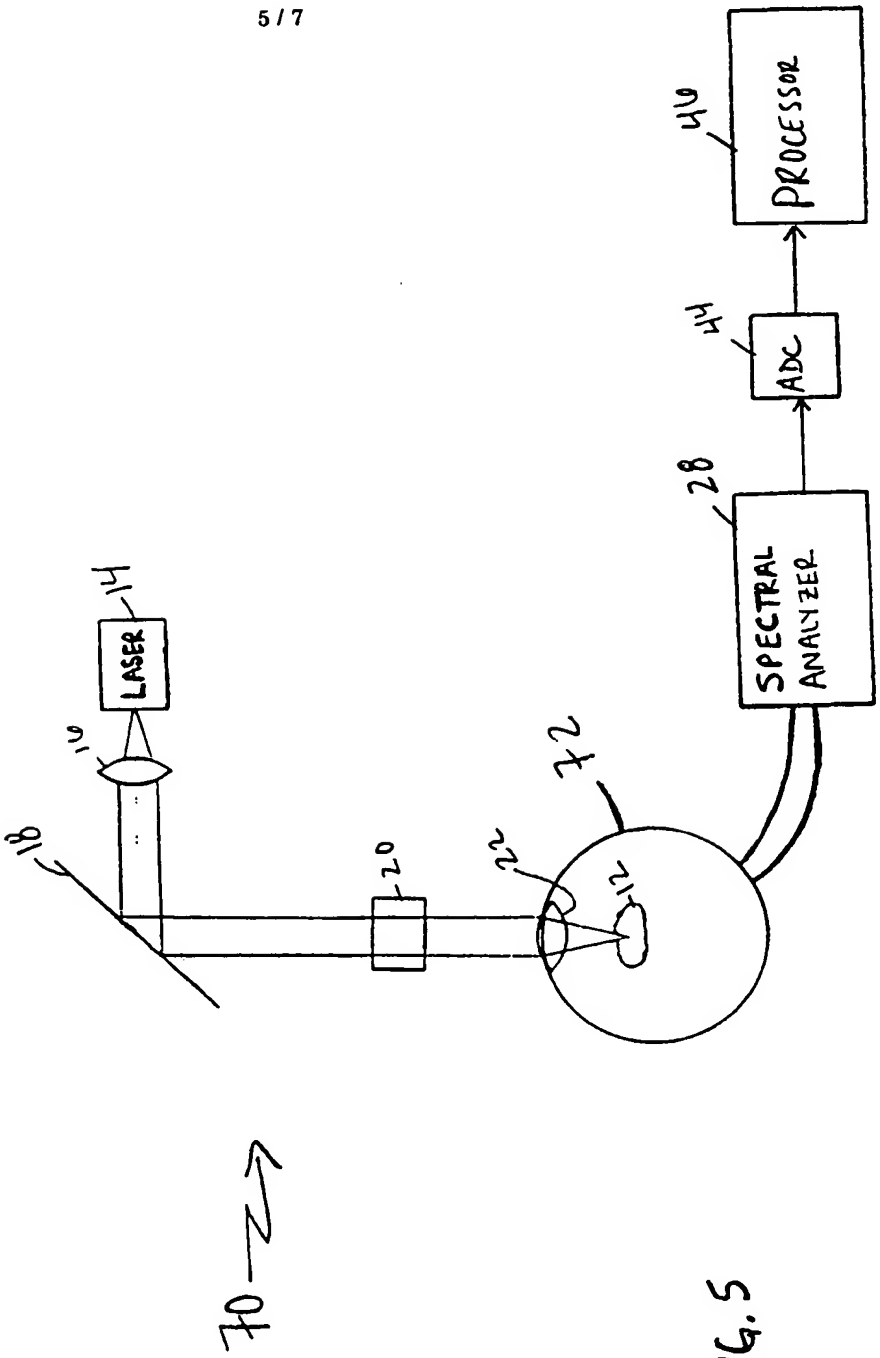

PROCESSOR
40
ADC
44
SPECTRAL ANALYZER
28
LASER
14
16
18
20
22
12
72
70

FIG. 5

FIG. 6

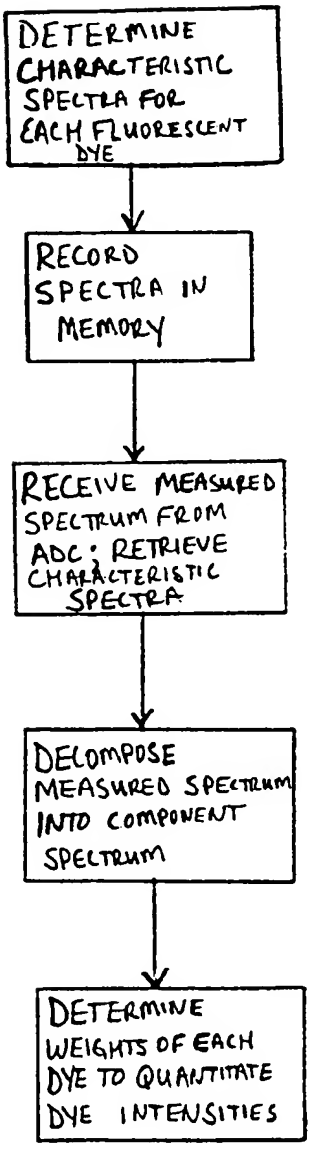

DETERMINE CHARACTERISTIC SPECTRA FOR EACH FLUORESCENT DYE
RECORD SPECTRA IN MEMORY
RECEIVE MEASURED SPECTRUM FROM ADC; RETRIEVE CHARACTERISTIC SPECTRA
DECOMPOSE MEASURED SPECTRUM INTO COMPONENT SPECTRUM
DETERMINE WEIGHTS OF EACH DYE TO QUANTITATE DYE INTENSITIES

FIG. 7a

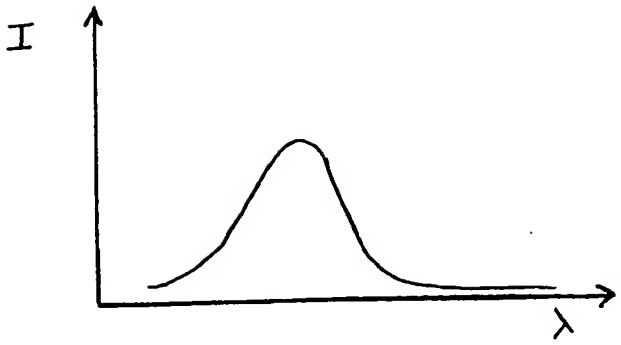



FIG. 7b

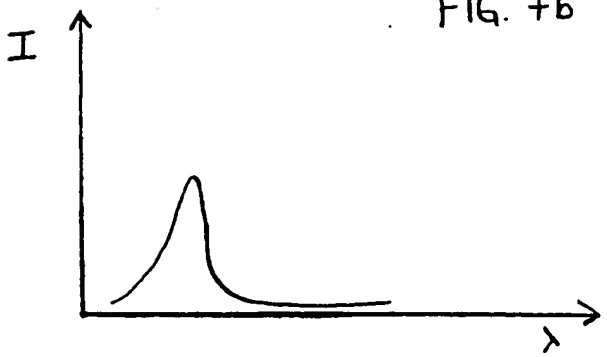



FIG. 7c

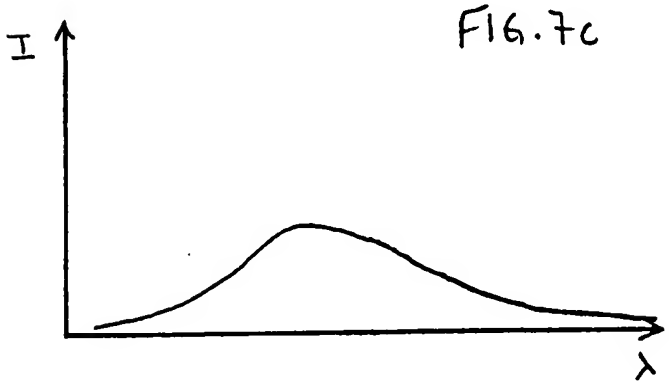
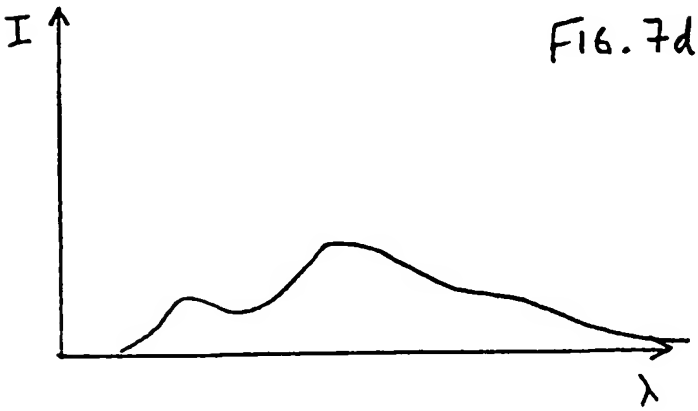



FIG. 7d



# INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/US 00/20591

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/64 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 96 22521 A (SOINI ERKKI ;HAENNINEN PEKKA (FI)) 25 July 1996 (1996-07-25) page 14; claims 1-3; figure 1 | 1-33 |
| A | US 5 891 738 A (SOINI ERKKI ET AL) 6 April 1999 (1999-04-06) column 15, line 40 - line 55 | 1-33 |
| A | US 5 674 743 A (ULMER KEVIN M) 7 October 1997 (1997-10-07) figure 9 | 1-33 |
| A | EP 0 916 981 A (MAX PLANCK GESELLSCHAFT) 19 May 1999 (1999-05-19) figure 3 | 1-33 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 28 November 2000 | 06/12/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Mason, W |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/US 00/20591

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 4 905 169 A (BUICAN TUDOR N ET AL)<br>27 February 1990 (1990-02-27)<br>figure 7 | 1-33 |
| A | US 5 117 466 A (BUICAN TUDOR N ET AL)<br>26 May 1992 (1992-05-26)<br>claim 1 | 1-33 |
| A | US 5 814 820 A (DONG CHEN-YUAN ET AL)<br>29 September 1998 (1998-09-29)<br>figure 5 | 1-33 |
| A | WO 99 37999 A (WISCONSIN ALUMNI RES FOUND)<br>29 July 1999 (1999-07-29)<br>figure 1 | 1-33 |

1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

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| US 5117466 A | 26-05-1992 | NONE | |
| US 5814820 A | 29-09-1998 | NONE | |
| WO 9937999 A | 29-07-1999 | AU 2339899 A | 09-08-1999 |
| | | GB 2349033 A | 18-10-2000 |